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Award Number: DAMD17-03-1-0077

TITLE: Development and Pre-Clinical Evaluation of a Novel Prostate-Restricted Replication Competent Adenovirus-AD-IU-1

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REPORT DATE: May 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2004	Annual (1 May 2003 - 30 Apr 2004)

4. TITLE AND SUBTITLE
Development and Pre-Clinical Evaluation of a Novel Prostate-Restricted Replication Competent Adenovirus-AD-IU-1

5. FUNDING NUMBERS
DAMD17-03-1-0077

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Recently we generated a prostate specific chemic promoter, called PSES, by combining the active prostate specific enhancers from PSA and PSMA genes which are prominently expressed in androgen independent prostate cancers. The goal of this research is to develop a novel therapeutic agent, Ad-IU-1, using PSES to control the replication of adenovirus and the expression of a therapeutic gene, herpes simplex thymidine kinase. Due to the size limitation of adenoviral vector, we need to shorten the size of PSES enhancer. In the past year, we have successfully shorten PSES from 513 bp to 407 bp and made a pAd-IU-1 cosmid vector as described in the original proposal. After releasing the adenoviral genome by Pac I digestion, pAd-IU-1 failed to produce an adenovirus. We believe that the size of the recombinant adenoviral genome is still too big to be packed. While we had a difficult time to make Ad-IU-1, we developed another PSES-E4 based replicative adenovirus and switched our viral construction strategy. By utilizing adenoviral E4 region, we made our Ad-IU-1, by putting E1a and E4 under the control of PSES and E1b and TK under the control of shortened PSES (called m6). We are currently characterizing Ad-IU-1.

14. SUBJECT TERMS
Transcriptional Regulation of Adenoviral Replication, Gene Therapy, PSES Chimeric Promoter, Tissue Restricted Replication Competent Adenovirus, Micropet Imaging

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION

Metastatic human prostate cancer (PC) is commonly treated by hormone, radiation, and/or chemotherapy. Inevitably, these patients will eventually relapse and develop androgen-independent disease with osseous metastasis. Since no effective therapy is presently available for the treatment of PC metastasis, we are developing a novel gene therapy modality for hormonal refractory prostate cancer based on a prostate-specific chemic promoter, PSES generated in my laboratory. In this study, we proposed to generate a herpes simplex virus thymidine kinase armed prostate restricted replicative adenovirus to treat androgen-independent prostate cancers. Specific Aim 1 intends to simplify and combine the most important enhancer elements from PSA and PSMA enhnacers/promoters to generate a strong and simple prostate-specific chimeric enhancer, sPSES. Specific Aim 2 will test whether sPSES retains prostate specific activity in an adenoviral vector. Specific Aim 3 will test whether sPSES can control adenoviral replication by controlling adenovirus E1a and E1b expression, and investigate how replication competent adenovirus eradicates prostate cancers by micro positron emission tomography (microPET) imaging.

BODY

Task 1. To generate a strong and simple prostate-specific enhancer. (Months 1-6):

a. Generate deletion consrtuct (Months 1-4). We have successfully deleted L2 and L5 in PSES, and replaced the 90 bp proximal region of PSME with simple AP-3 binding site. These manipulations reduced the size of PSES from 513 bp to 407 bp. The simplified version of PSES is called m6.

b. Test the tissue-specificity of new deletion constructs (Months 3-6). The tissue specific activity of m6 has been tested in several cell lines. Figure 1 demonstrated that m6 retained a strong prostate specificity.

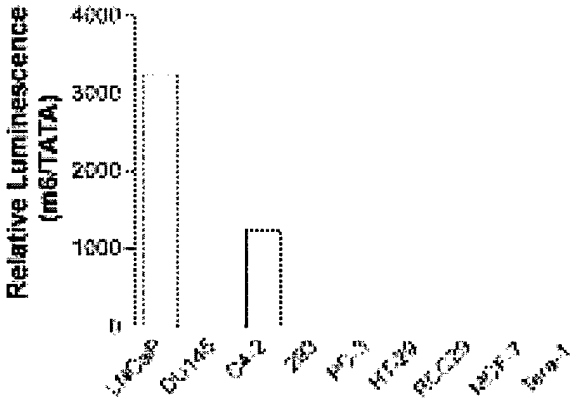


Figure 1. pGL3/m6/TATA (0.5 μg) was transfected into various cell lines (2 x 10^5 cells for each). After 2 days, cells were harvested, lysed with passive lysis buffer (Promega) and analyzed for luciferase activities. This experiment was conducted in the absence of androgen. The luciferase activity was determined by being divided by the basal activity represented by transfection of pGL3/TATA. pGL3/m6/TATA is avtive only in PSA/PSMA postivie LNCaP and C4-2 cells.

Task 2. To test tissue-specificity of the modified PSES chimeric enhancer, m6, in an adenoviral vector (Months 7-24):

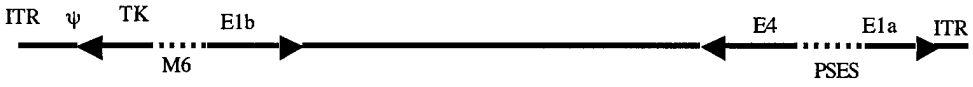
a. Construct Ad-m6-Luc (Months 7-12). Ad-m6-Luc has been generated. We are in the process of amplifying the virus for next step testing.

b. Test the tissue-specificity of Ad-sPSEC-Luc in tissue culture cells (Months 13-18). Ongoing.

c. Test the tissue-specificity of Ad-sPSEC-Luc in vivo (Months 19-22). Ongoing.

Task 3. Investigate the capability of sPSEC to drive adenovirus replication in a prostate cancer-specific manner (Months 13-36):

a. Construct Ad-IU-1 (Months 7-16). The first attempt to make Ad-IU-1 according to the original plan has failed due to a size limitation of adenoviral vector. We have changed our construction strategy and made Ad-IU-1 as illustrated below.



b. In vivo test tissue-specific expression of TK, E1a and E1b proteins in Ad-IU-1 infected cells (Months 17-20). On going.

c. In vitro test the tissue specific replication of Ad-IU-1 (Months 19-22). On going.

d. In vitro test the therapeutic efficacy of Ad-IU-1 (Months 20-23). On going.

e. In vivo test the therapeutic efficacy of Ad-IU-1 (Months 22-36).

f. MicroPet image C4-2 tumors (Months 9-20). It has been finished and published (see Reportable Uutcomes section). In general, microPET imaging of tumor is less sensitive than PSA assay. The reviewers of this proposal also pointed out this problem.

g. MicroPET image the therapeutic effect of Ad-IU-1 (Months 21-36). We have made 8-[^{18}F]-fluoropenciclovir for TK microPET imaging study (see Reportable Uutcomes section).

KEY RESEARCH ACCOMPLISHMENTS

1. Successfully shorten PSES as planned and demonstrate the prostate specific activity of the shortened form of PSES, m6.

2. Successfully construct Ad-m6-Luc and Ad-IU-1.

3. Have tested [^{11}C]-choline and [^{18}F]-FDG microPET imaging in prostate cancer nude mice model.

4. Successfully synthesize 9-(4-[18F]fluoro-3-hydroxymethylbutyl)guanine ([18F]FHBG) for microPET imaging Ad-IU-1 activity.

REPORTABLE OUTCOMES

Some of the results of this study has been published.

1. Q-H. Zheng, **T.A. Gardner,** S.P. Raikwar, C. Kao, K.L. Stone, T.D. Martinez, B.H. Mock, X. Fei, J-Q. Wang, G.D. Hutchins: [11C]Choline as a PET biomarker for assessment of prostate cancer tumor models. *Bioorg. Med. Chem.* **2004**, *12*, 2887-2893.

2. J-Q. Wang, Q-H. Zheng, X. Fei, X. Liu, **T.A. Gardner,** C. Kao, S.P. Raikwar, B.E. Glick-Wilson, M.L. Sullivan, B.H. Mock, G.D. Hutchins: An improved total synthesis of PET HSV-tk gene expression imaging agent 9-[(3-[18F]fluoro-1-hydroxy-2-propoxy)methyl]guanine ([18F]FHPG). *Syn. Commun.* **2004**, *34(5)*, 917-932.

3. Q-H. Zheng, J-Q. Wang, X. Liu, X. Fei, B.H. Mock, B.E. Glick-Wilson, M.L. Sullivan, S.P. Raikwar, **T.A. Gardner,** C. Kao, G.D. Hutchins: An improved total synthesis of PET HSV-tk gene reporter probe 9-(4-[18F]fluoro-3-hydroxymethylbutyl)guanine ([18F]FHBG). *Syn. Commun.*, **2004**, *34(4)*, 689-704.

CONCLUSIONS

In this past year we have successfully shortened PSES as planned and demonstrated the prostate specific activity of the shortened form of PSES, m6. Ad-m6-Luc and Ad-IU-1 have been constructed. We are on the process of characterizing these two viruses. We have tested the sensitivity of using [^{11}C]-choline and [^{18}F]-FDG microPET imaging technology to monitor prostate cancers in nude mice model. It is unlikely that we can use these microPET imaging technique to monitor prostate bone metastasis due to its poor sensitivity. We have successfully synthesize 9-(4-[18F]fluoro-3-hydroxymethylbutyl)guanine ([18F]FHBG) for microPET imaging Ad-IU-1 activity.

REFERENCES

APPENDICES